# Congenital adrenal hyperplasia: diagnostic advances

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NEWBORN SCREENING

# Congenital adrenal hyperplasia: Diagnostic advances

**T. Torresani · Anna Biason-Lauber**

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**Summary** Congenital adrenal hyperplasia is a group of autosomal recessive disorders resulting from the deficiency of one of the five enzymes required for the synthesis of cortisol in the adrenal cortex. The most frequent is steroid 21-hydroxylase deficiency, accounting for more than 90% of cases. Much has been learned about the genetics of the various clinical forms of 21-hydroxylase deficiency, and correlations between the genotype and the phenotype have been studied extensively. Gene-specific diagnosis is now feasible and neonatal screening and prenatal treatment have been widely implemented. This discussion will be limited to the most common form of congenital adrenal hyperplasia, with focus on the diagnostic advances in this disease.

**Abbreviations**

| | |
|---|---|
| 11β-OHD | 11β-hydroxylase deficiency |
| 17-OHP | 17-hydroxyprogesterone |
| 21-OHD | 21-hydroxylase deficiency |
| CAH | congenital adrenal hyperplasia |
| DBS | dried blood spots |
| DSD | disorder of sex development |
| LDR | ligase detection reaction |
| MLPA | multiplex ligation-dependent probe amplification |

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## Introduction

Congenital adrenal hyperplasia (CAH) is one of the most frequent inborn endocrine disorders; it comprises autosomal recessive disorders of cortisol biosynthesis in the adrenal gland caused by various enzyme deficiencies. The consequent compensatory rise of ACTH production causes hyperplastic growth of the adrenal glands. Blocks of the initial steps of the steroidogenic pathway impair the production of all the three types of steroids, i.e. mineralocorticoids, glucocorticoids and sex hormones, causing abnormalities in the salt–water homeostasis and in sexual differentiation.

21-Hydroxylase deficiency (21-OHD) accounts for most cases of CAH (80–90%, depending on the ethnic group) (Miller 1994; Miller and Levine 1987). Clinical consequences of 21-OHD arise from overproduction of androgens. Affected females with the *classic* 21-OHD are born with ambiguous genitalia. Postnatally, untreated patients of both sexes manifest rapid somatic growth with accelerated skeletal maturation, early closure of the epiphyses, and short adult stature. Other symptoms include excessive pubic and body hair and decreased fertility. Seventy-five per cent of patients with classic 21-OHD also have reduced synthesis of aldosterone with salt loss. Patients with *nonclassic* disease are born without symptoms of prenatal androgen exposure. Subsequently they may remain asymptomatic or may develop signs of androgen excess.

Deficiency of 21-hydroxylase is inherited as an autosomal recessive trait closely linked to the HLA major histocompatibility complex on the short arm of chromosome 6. While classic 21-OHD is found in about 1 in 16 000 births, nonclassic deficiency is far more frequent, occurring in up to 3% of persons among certain ethnic groups (Speiser et al 1985). The clinical presentation of patients with CAH is heterogeneous and depends on the type of gene mutation as well as on the sex of the patient (Hughes 1998). Some newborns with CAH may thus present without clinical signs or symptoms postnatally and in these the diagnosis of CAH obviously cannot be made on clinical basis. The unrecognized and thus untreated disease may entail life-threatening salt-wasting crisis in the newborn period and lead to morbidity later in life, including precocious puberty and short stature. Thus it is evident that implementation of a programme that fulfills the feasibility criteria of neonatal screening is beneficial in preventing delayed diagnosis of CAH and its associated morbidity and mortality sequelae (Honour and Torresani 2001; Pang et al 1977).

The most feasible biochemical marker for the diagnosis of CAH is 17-hydroxyprogesterone (17-OHP), the steroid metabolite lying just upstream the block (Fig. 1). Screening for CAH by measuring 17-OHP levels in dried blood spots (DBS) of newborns was incorporated in the Swiss neonatal screening programme for metabolic and endocrine diseases at the end of 1992. It can detect most forms of 21-OHD and some cases of 11β-hydroxylase deficiency (11β-OHD). All other, far less frequent, enzyme deficiencies of the adrenal gland leading to CAH cannot be found with this screening parameter.

Comparatively, all other adrenal enzyme deficiencies leading to CAH are relatively rare. Briefly, in lipoid adrenal hyperplasia no conversion of cholesterol to any steroid takes place. This rare cause of CAH is characterized by salt loss and disorder of sex development (DSD) in XY individuals. In XX subjects internal and external genitalia are female, and the syndrome cannot clinically be separated from congenital adrenal hypoplasia . The molecular bases of such a defect have recently been clarified as mutations in the steroidogenic acute response protein (StAR). 17α-Hydroxylase deficiency, leads to 46, XY DSD due to the lack of precursors for testosterone. In XX individuals, there is primary amenorrhoea and absent development of oestrogenic secondary sexual characteristics. Both sexes display hypertension and hypokalaemic alkalosis due to accumulation of mineralocorticoid precursors, which do not need 17α-hydroxylation for their synthesis. Adrenal hyperplasia and glucocorticoid deficiency are less marked than in the other types of CAH because of the ability of corticosterone to suppress ACTH. Male patients affected by CAH due to 3β-hydroxysteroid dehydrogenase (3βHSD) deficiency display incomplete prenatal masculinization due to

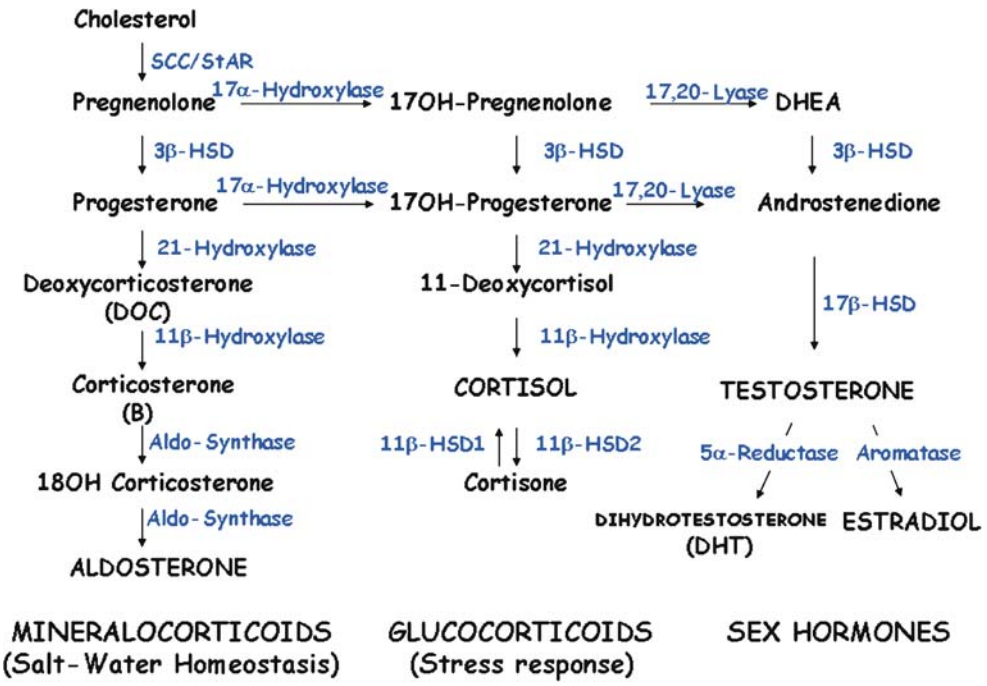


**Fig. 1** Steroidogenic pathway. Steroidogenic acute response protein (StAR) is involved in cholesterol transport through mitochondrial membrane and not in an enzymatic step

the impaired synthesis of bioactive androgens, and salt loss due to lack of mineralocorticoid. XX subjects have normal female external genitalia or mild virilization due to the action of the weak androgen. Steroid 11β-OHD, which is responsible for 10–20% of cases of CAH, produces symptoms of androgen excess similar to those in 21-OHD. The blocked enzymatic step also results in accumulation of 11-deoxycorticosterone, which has mineralocorticoid activity, leading to hypertension in untreated patients.

## Biochemistry

Steroid 21-hydroxylase (CYP21, also termed CYP21A2 and P450c21) is a cytochrome P450 enzyme located in the endoplasmic reticulum. It catalyses the conversion of 17-OHP to 11-deoxycortisol, a precursor of cortisol, and the conversion of progesterone to deoxycorticosterone, a precursor of aldosterone (Fig. 1).

Patients with 21-OHD cannot synthesize cortisol efficiently, and as a result, the adrenal cortex is stimulated by corticotropin and overproduces cortisol precursors. Some of these precursors are used for the biosynthesis of sex hormones, which may cause signs of androgen excess, including ambiguous genitalia in newborn girls and rapid postnatal growth in both sexes. Concomitant aldosterone deficiency may lead to salt wasting with consequent failure to thrive, hypovolaemia and shock.

## Clinical presentation

Different phenotypes are observed. A severe form with a concurrent defect in aldosterone biosynthesis (salt-wasting type) and a form with apparently normal aldosterone biosynthesis (simple virilizing type) are called classic 21-OHD. There is also a mild, nonclassic form that may be asymptomatic or associated with signs of postnatal androgen excess (White and Speiser 2000).

Classic 21-OHD is detected in approximately 1 in 16 000 births in most populations (Therrell 2001). In Switzerland, the disease is detected in approximately 1:10 000 with a carrier frequency of 1:50, which is in agreement with figures of other European countries. The nonclassic form occurs in approximately 0.2% of the general white population but is more frequent (1–2%) in certain populations, such as Jews of Eastern European origin (Speiser et al 1985). The lower general frequency is similar to that estimated on the basis of *CYP21* genotyping of newborns in New Zealand (0.3%) (Fitness et al 1999).

### Salt wasting

Approximately 75% of patients with classic 21-OHD have severely impaired 21-hydroxylase activity and therefore cannot adequately synthesize aldosterone. Elevated levels of 21-hydroxylase substrates—mostly 17-OHP—may act as mineralocorticoid antagonists, exacerbating the effects of aldosterone deficiency (Oelkers 1996). Since aldosterone regulates sodium homeostasis, renal sodium excretion in untreated patients is excessive and can result in hypovolaemia and hyperreninaemia. Such patients cannot excrete potassium efficiently and are prone to hyperkalaemia, especially in infancy. Cortisol deficiency in these patients contributes to poor cardiac function, poor vascular response to catecholamines, a decreased glomerular filtration rate, and increased secretion of antidiuretic hormone (Lamberts et al 1997). Thus, cortisol and aldosterone deficiency together cause hyponatraemic dehydration and shock in inadequately treated patients. Moreover, since the development of the adrenal medulla is in part dependent on glucocorticoids, patients with salt-wasting 21-OHD may also have catecholamine deficiency, potentially aggravating shock (Merke et al 2000).

Patients with the salt-wasting form are identified through the measurement of serum electrolytes, aldosterone and plasma renin and the finding of expected abnormalities, hyperkalaemia, low levels of aldosterone and hyperreninaemia.

### Ambiguous genitalia

Girls with classic 21-OHD are exposed *in utero* to high levels of adrenal androgens from approximately the seventh week of gestation. Thus, such girls have ambiguous external genitalia. The uterus, Fallopian tubes, and ovaries are normally formed, but there is no development of the Wolffian duct. In contrast, affected boys have no overt signs of the disease except variable and subtle hyperpigmentation of the scrotum and penile enlargement.

### Postnatal virilization

In untreated or poorly treated patients, long-term exposure to high levels of sex hormones promotes rapid somatic growth and advanced skeletal age, which leads to premature epiphyseal fusion and low adult height. Pubic and axillary hair may develop early. Clitoral growth may continue in girls. Young boys may have penile growth despite having small testes, since the androgens are adrenal in origin. Long-term exposure to

androgens may activate the hypothalamic–pituitary–gonadal axis, causing central precocious puberty.

### Reproductive function

In girls with any form of 21-OHD, signs of reproductive abnormalities, such as oligomenorrhoea or amenorrhoea, may develop in adolescence (Barnes et al 1994; Deneux et al 2001). The issue of fertility is mainly related to psychosocial adjustment. Women with classic salt-wasting or simple virilizing disease who were born and treated in the early days tend to avoid heterosexual relationships, especially if the surgical correction of the external genitalia was inadequate or androgen levels were constantly elevated (Mulaikal et al 1987).

As surgical, medical, and psychological treatments have improved, more women with 21-OHD have successfully completed pregnancies and given birth, most by Caesarean section (Lo and Grumbach 2001; Premawardhana et al 1997). About 80% of women with simple virilizing disease and approximately 60% of those with the severe salt-wasting form are fertile.

Compared with affected women, affected men have fewer problems with reproductive function, specifically gonadal function. Most have normal sperm counts and are able to father children (Cabrera et al 2001; Urban et al 1978). One relatively common form of gonadal abnormality in affected males is the development of testicular adrenal rests, detectable by sonographic imaging before they become palpable (Stikkelbroeck et al 2001). Such tumours have been detected even in childhood (Murphy et al 2001), suggesting that the search for them should begin no later than adolescence. In males with salt wasting, testicular rest tissue may be accompanied by deficient spermatogenesis despite treatment. Infertility can be circumvented by intracytoplasmic sperm injection (Walker et al 1997). These tumours are always benign and orchidectomy is usually not necessary. Proper medical treatment consists of pituitary suppression with dexamethasone, since the tumours are usually responsive to corticotropin.

### Patients with simple virilizing 21-hydroxylase deficiency

Patients with simple virilizing 21-OHD do not synthesize cortisol efficiently, but aldosterone secretion is sufficient to maintain sodium balance. Whereas the disease is usually diagnosed in female patients shortly after birth due to genital ambiguity, the diagnosis is often delayed for several years in male patients. Without newborn screening, affected boys are usually identified when signs of androgen excess develop. Later diagnosis is associated with greater difficulty in achieving hormonal control, abnormal tempo of puberty, and short stature.

### Patients with nonclassic disease

Patients with nonclassic 21-OHD produce normal amounts of cortisol and aldosterone at the expense of mild-to-moderate overproduction of sex hormone precursors. A few nonclassic cases are detected by newborn-screening programmes, but most are missed because of the relatively low baseline levels of 17-OHP (Balsamo et al 1996; Tajima et al 1997; Therrell et al 1998). Hirsutism is the single most common symptom at presentation in approximately 60% of symptomatic women, followed by oligomenorrhoea (54%) and acne (33%) (Moran et al 2000). Thus, nonclassic 21-OHD and polycystic ovary syndrome may present in similar ways.

### Heterozygotes

Patients who are heterozygous for *CYP21A2* mutations often have slightly higher 17-OHP levels after adrenal stimulation than do unaffected subjects. Although it has been suggested that heterozygotes might be more likely to have signs of androgen excess than would genetically unaffected subjects, case–control studies do not support this concept (Knochenhauer et al 1997).

## Diagnosis

### Screening

Classic 21-OHD is characterized by markedly elevated serum levels of 17-OHP, the main substrate for the enzyme. Basal serum 17-OHP values measured by radioimmunoassay after extraction usually exceed 300 nmol/L in infants with classic CAH, whereas the levels in normal newborns are below 3 nmol/L. This difference makes it possible to screen newborns for the disorder with the use of dried blood spots on filter paper. Screening minimizes delays in diagnosis, especially in male patients, and reduces morbidity and mortality from adrenal crises. One major problem in CAH screening is posed by the fact that most premature infants, especially those with gestational ages of less than 31 weeks, have elevated 17-OHP levels without having inborn errors in steroid biosynthesis. This event is most likely due to physiologically

delayed expression of the enzyme 11β-hydroxylase (Hingre et al 1994). Elevation of 17-OHP levels in both term and preterm babies can be also due to illness, poor kidney or liver function, stress and sampling before 48 h of life. It has therefore become common practice to have different algorithms in CAH screening, one for premature and one for term babies (Figs. 2 and 3).

Another factor contributing to false elevations of measured 17-OHP is the limited specificity of some antisera used in the immunoassays of 17-OHP. Particularly important is the cross-reactivity with 17-hydroxypregnenolone and its sulfate (up to 8% cross-reactivity), compounds that tend to be rather high in newborns (due to possible inhibition of 3βHSD by the maternal oestrogens).

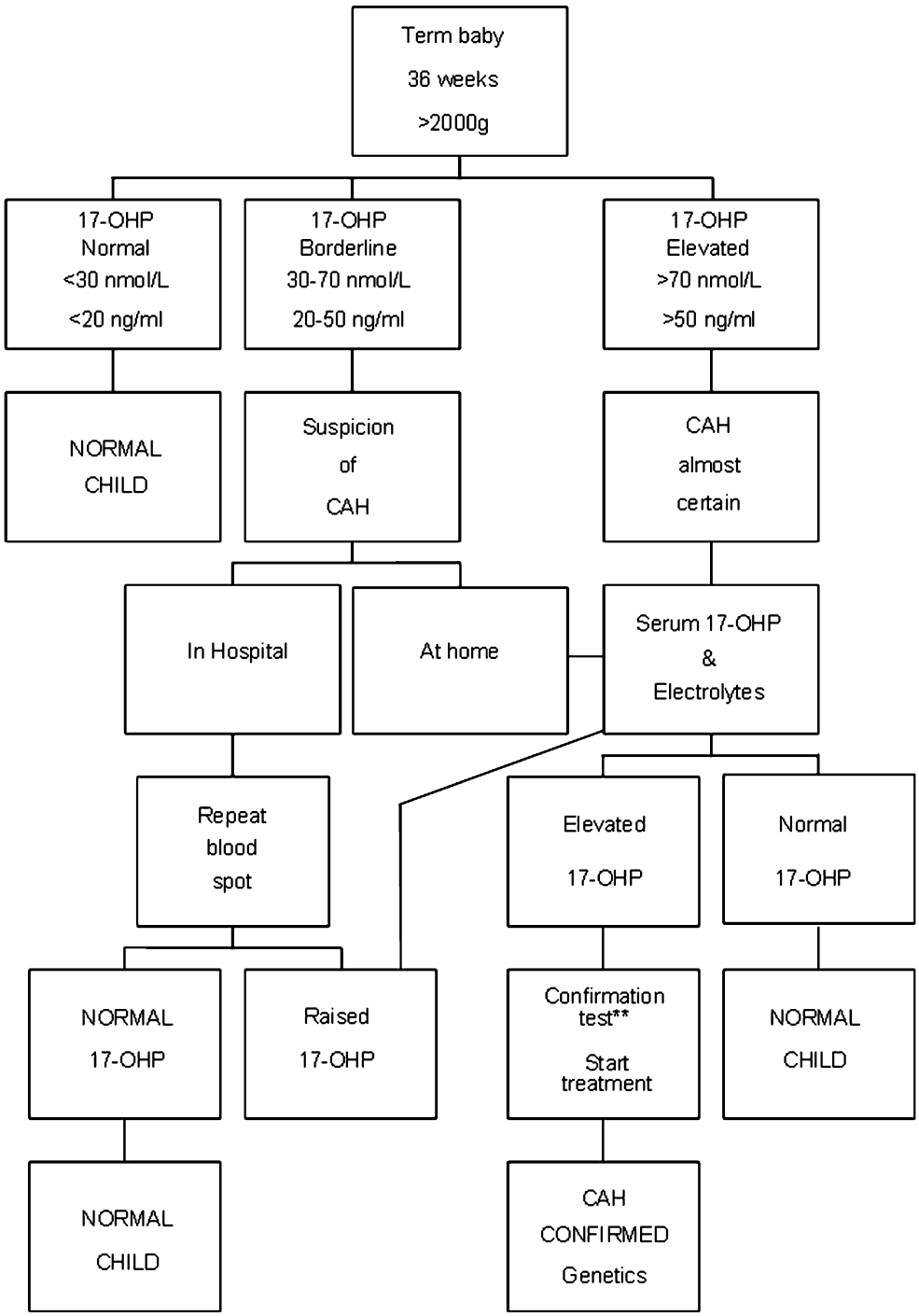


**Fig. 2** Example of a possible screening flowchart for term babies

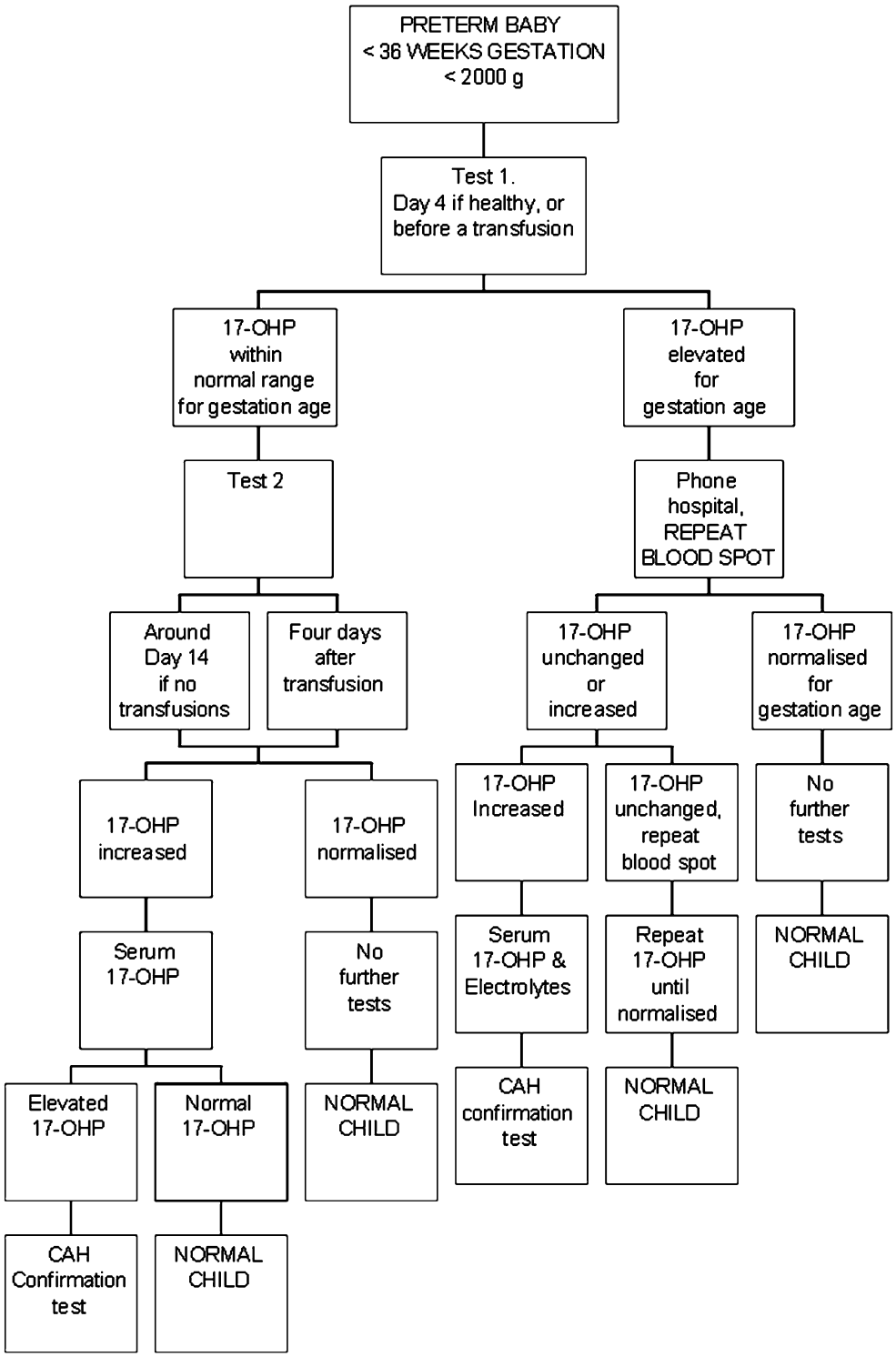


**Fig. 3** Example of a possible screening flowchart for premature newborns

The high rate of false-positive values not only increases the real cost of screening but also causes psychological distress to the parents. Delays in accurate diagnosis can lead either to unnecessary steroid therapy or to failure to institute therapy in a timely manner. Many screening programmes have therefore started to perform routinely two tests in premature infants with the aim of avoiding unnecessary recalls. This measure is easy to implement and has proved to be effective in reducing the rate of false-positive screening results and in improving the positive predictive value of an elevated 17-OHP concentration (Steigert et al 2002).

To further improve accuracy, some screening programmes have set reference levels for serum 17-OHP in infants that are based on weight or gestational age (Fig. 4) (Torresani et al 1994; van der Kamp et al 2005).

Recently it has been suggested that measurement of 17-OHP by tandem mass spectrometry may improve both the sensitivity and the specificity of screening. Tandem mass spectrometry (LC-MS/MS) might constitute a reliable second-tier testing for CAH (Janzen et al 2007; Minutti et al 2004) due to its high ability to precisely identify metabolites (specificity close to 100%) and its capability to simultaneously recognize several metabolites for the identification of defects other than 21-OHD. Nevertheless, MS/MS cannot yet be considered as an alternative for primary 17-OHP measurement in a mass screening programme, mainly because of its low sensitivity, the necessity for pretreatment of the sample (extraction) , the need of preseparation of the metabolites (via gas chromatography or liquid chromatography) and a relative long time per run (up to 12 min, depending on the available instrumentation). The utility of MS/MS measurement as a second-tier method for confirming elevated levels of 17-OHP has in any case already demonstrated its value (Janzen et al 2007).

In recent years, molecular diagnosis has been applied to confirm a diagnosis of CAH at the DNA level. The advantages of this method as second-tier testing rely on its high specificity. The recent report of a 3βHSD (HSD3B2) deficiency identified by screening (Nordenstrom et al 2007) indicates the possibility of recognizing this deficiency in the neonatal period, which does not seem to be the case for 11β-OHD (CYP11B1).

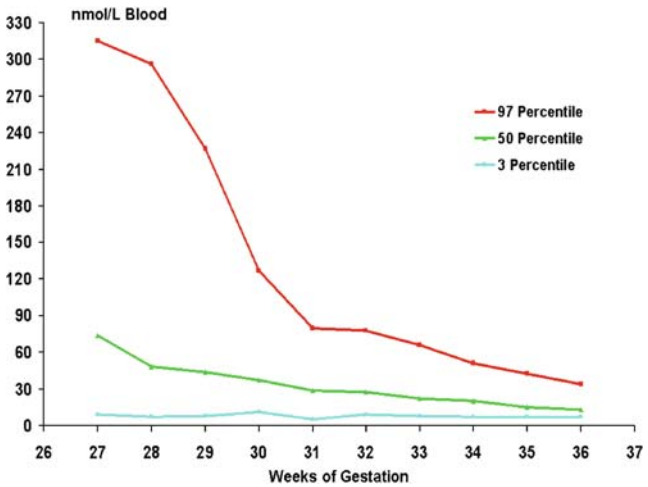


**Fig. 4** Normal values (expressed in centiles) of 17-hydroxyprogesterone related to gestational age (gestational week)

Other diagnostic procedures

The gold standard for differentiating 21-OHD from other steroidogenic enzyme defects is the corticotropin (Synachten) stimulation test, performed by injecting a 0.125 mg or 0.25 mg bolus of ACTH and measuring baseline and stimulated levels of 17-OHP. Blood samples are obtained at baseline and 60 min after the administration of ACTH. Except for premature infants, there are no age-related differences in the criteria for the diagnosis of 21-OHD on the basis of 17-OHP levels.

The severity of hormonal abnormalities depends on the type of 21-OHD. Patients with salt-wasting disease have the highest 17-OHP levels (up to 3000 nmol/L after corticotropin stimulation), followed by patients with simple virilizing disease, who usually have somewhat lower levels (300–1000 nmol/L). Patients with nonclassic disease have smaller elevations (50–300 nmol/L), especially in the newborn period. Random measurements of basal serum 17-OHP levels are often normal in patients with nonclassic disease unless the values are obtained in the early morning. Thus, the diagnosis is most reliably made by measuring the patient's response to corticotropin stimulation.

Other hormones whose levels are usually elevated in patients with 21-OHD include progesterone, androstenedione and, to a lesser extent, testosterone. An atypical steroid, 21-deoxycortisol, is also elevated but is not routinely assayed. Mutation analysis can confirm the diagnosis and is used in some newborn-screening programmes.

## Genetics

Mutations in the *CYP21* (*CYP21A2*) gene, which is located in the highly polymorphic HLA histocompatibility complex on chromosome 6p21.3 along with a pseudogene, *CYP21A1P* (*CYP21P*), are responsible for causing 21-OHD. Although *CYP21A2* and *CYP21P* have 98% nucleotide-sequence identity, the latter has accumulated several mutations that totally inactivate its gene product. These include an 8 bp deletion in exon 3, a frameshift in exon 7, and a nonsense mutation in exon 8 (Fig. 5). Additional mutations in *CYP21P* affect messenger RNA (mRNA) splicing or amino acid sequence. Most mutations causing 21-OHD arise from two types of recombination between *CYP21A2* and *CYP21P*. Approximately 3/4 represent deleterious mutations found in the pseudogene that are transferred to *CYP21* during mitosis by a process termed 'gene conversion'. About 20% are meiotic recombinations that delete a 30 kb

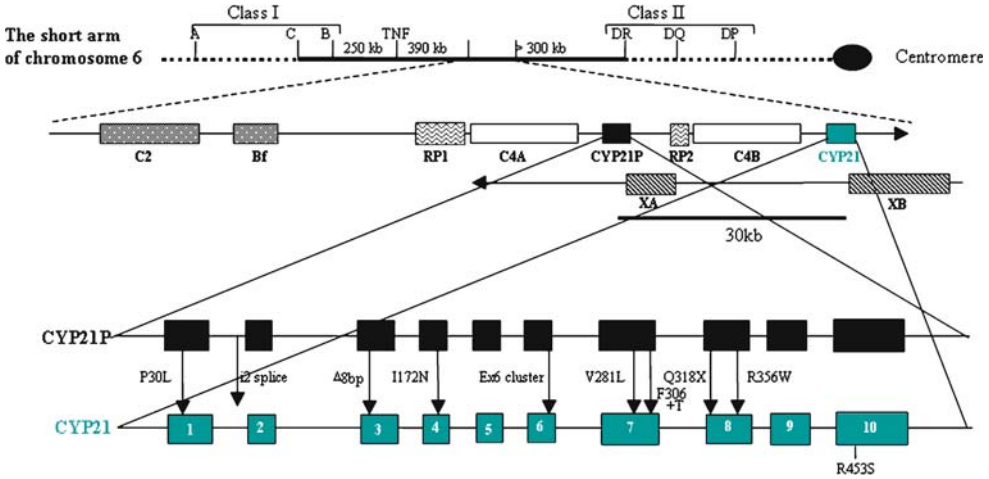


**Fig. 5** Organization of the genomic region containing the 21-hydroxylase active gene. Arrows indicate direction of transcription. CYP21, 21-hydroxylase gene; CYP21P, 21-hydroxylase pseudogene; C4A and C4B, genes encoding the fourth component of serum complement; RP1, gene encoding a putative nuclear protein of unknown function; RP2, truncated copy of RP1; XB, tenascin–X gene; XA, truncated copy of XB. These two sequences are on the opposite chromosomal strand

gene segment that encompasses the 3′ end of the *CYP21P* pseudogene, all of the adjacent *C4B* complement gene, and the 5′ end of *CYP21A2*, producing a nonfunctional chimeric pseudogene.

The search for mutations is made easier since the great majority (up to 95%) of the mutant alleles will carry one of the 10 pseudogene mutations. On the other hand, the high degree of sequence identity between the active gene and the pseudogene renders the specific amplification of the *CYP21A2* active gene rather challenging. The choice of primers hybridizing exclusively to sequences of the active *CYP21A2* gene (e.g. in exon 3 where the active gene contains 8 bp that are deleted in the pseudogene) helped to overcome this hurdle.

Several new methods are currently used in the molecular analysis of the *CYP21A2* gene.

## Large deletions

Southern blot analysis will reveal many aberrations but will not always detect small deletions and is not a simple to perform routine technique. Well-characterized deletions and amplifications can be detected by PCR. However, the exact breakpoint sites of most deletions have not been determined. Furthermore, the number of different deletions is becoming prohibitively large.

### *Multiplex ligation-dependent probe amplification* (*MLPA; MRC Holland*) (*Schouten et al* 2002)

Deletions and amplifications of (part of) a gene will usually not be detected by sequence analysis of PCR-amplified gene fragments as a normal copy is still present. Analysis by MLPA is a suitable alternative that is also capable of detecting new deletions and amplifications.

With MLPA, it is possible to perform a multiplex PCR reaction in which up to 45 specific sequences are simultaneously quantified. Amplification products are separated by sequence type electrophoresis. As only one pair of PCR primers is used, MLPA reactions result in a very reproducible gel pattern with fragments ranging from 130 to 490 bp. Comparison of this gel pattern with that obtained with a control sample indicates which sequences show an aberrant copy number (Fig. 6).

## Other mutations

### *Ligase detection reaction* (*LDR*) (*Day et al* 1995)

An equimolar mixture of two detecting (or allele-specific) oligonucleotides and one common oligonucleotide is hybridized to denatured PCR-amplified targets. The detecting oligonucleotides anneal immediately adjacent to the 5′-end of the common oligonucleotide, resulting in the formation of a short DNA duplex containing a nick at the junction site between the primers.

The two detecting primers are in competition for hybridization to the denatured target, and depending upon which of the detecting oligonucleotide has hybridized, the 3′-end of the allele-specific primer will have a perfect match or will contain a single base mismatch. If there is a match, then the junction

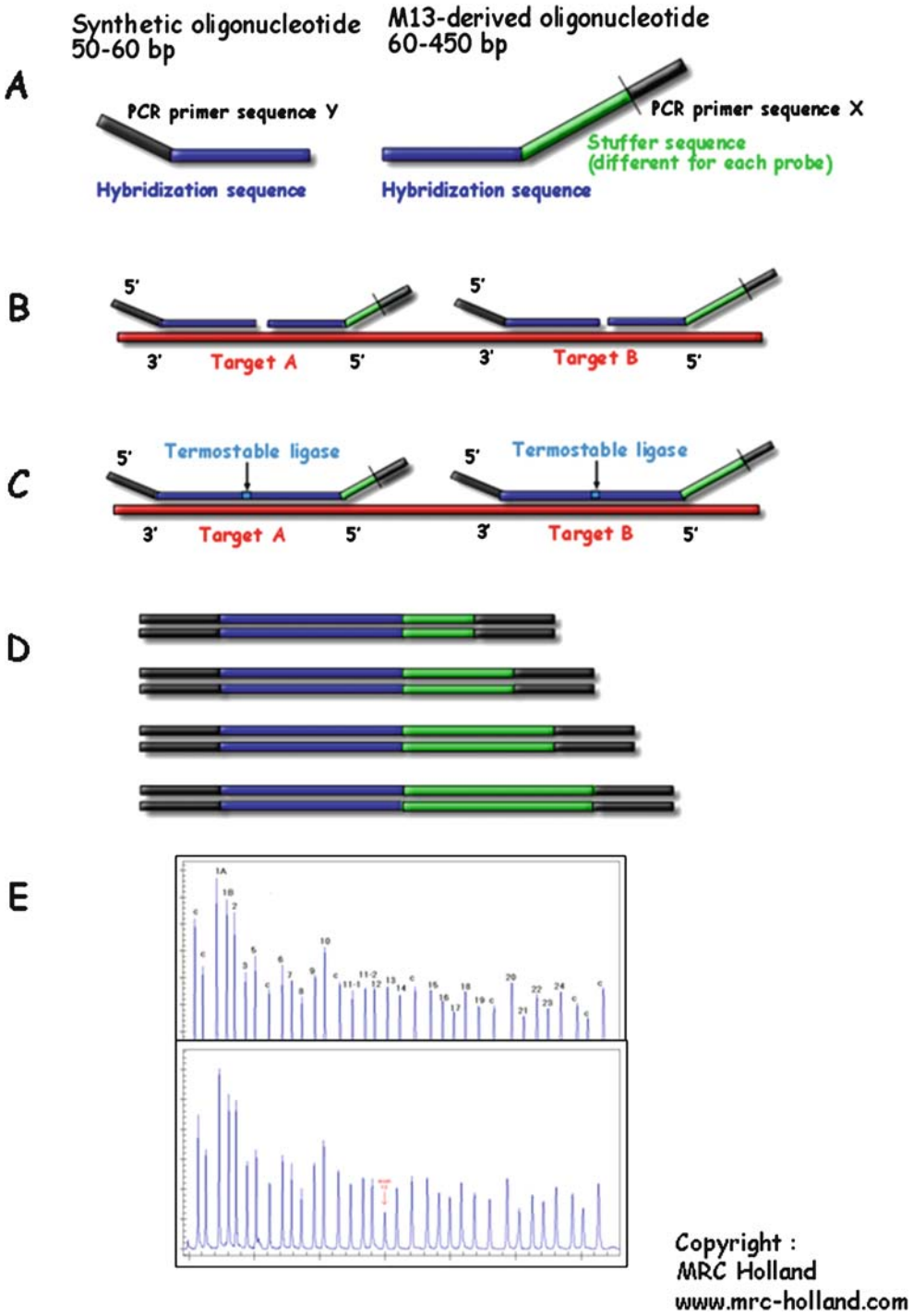


**Fig. 6** Multiplex ligase-dependent probe amplification: general scheme of method (copyright MRC Holland, from Schouten et al 2002; www.mrc-holland.com). (**A**) MLPA probes. (**B**) The MLPA probe mix is added to denatured genomic DNA. The two parts of each probe hybridize to adjacent target sequences. (**C**) Probes are ligated by a thermostable ligase. (**D**) A universal primer pair is used to amplify all ligated probes. The amplification product of each probe has a unique length. (**E**) Separation and quantification by capillary electrophoresis. Each peak is the amplification product of a specific probe. Samples are compared with a control sample. A difference in relative peak height or peak area indicates a copy number change of the probe target sequence

between the detecting and the common primers will be covalently sealed by DNA ligase.

We chose to differentiate the multiplex LDR products on the basis of length and labelling. This was achieved by synthesizing LDR oligonucleotides with synthetic poly(dA) tails such that each ligation product has a unique length, two nucleotides different from that of any other ligation product. At each gene

conversion site the CYP21P product was always two bases longer than the corresponding CYP21A2 product. The CYP21A2 products were each four bases longer than the previous going from the 5′ to 3′ end of the gene (Fig. 7). An example of an output of this analysis is depicted in Fig. 8.

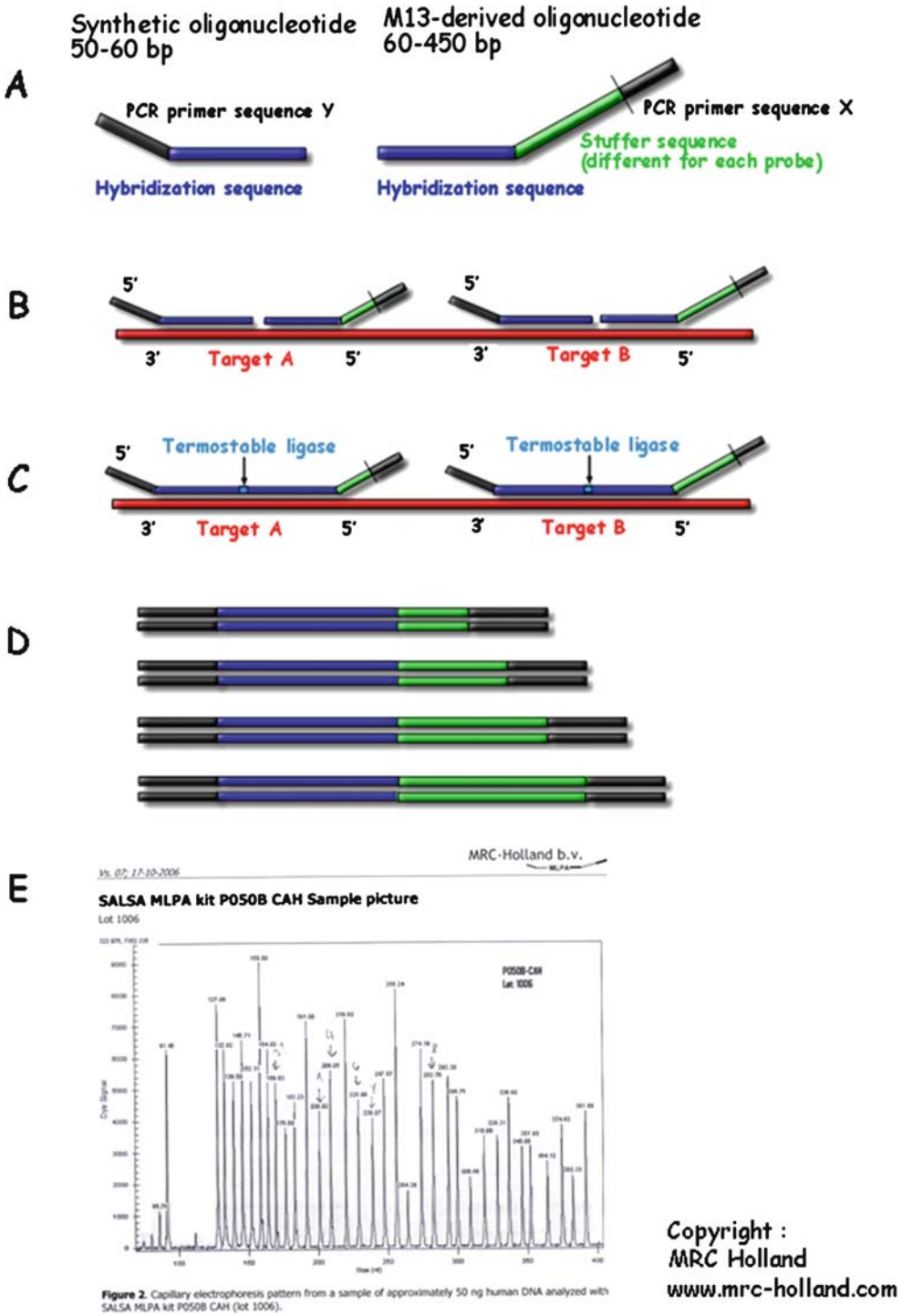


**Fig. 7** Ligase detection reaction: principle. Detecting (or allele-specific) oligonucleotides and one common oligonucleotide are hybridized to denatured PCR-amplified targets. The detecting oligonucleotides anneal immediately adjacent to the 5′-end of the common oligonucleotide, resulting in the formation of a short DNA duplex containing a nick at the junction site between the primers. If there is a match, then the junction between the detecting and the common primers will be covalently sealed by DNA ligase. We chose to differentiate the multiplex LDR products on the basis of length and labelling (see text for further details)

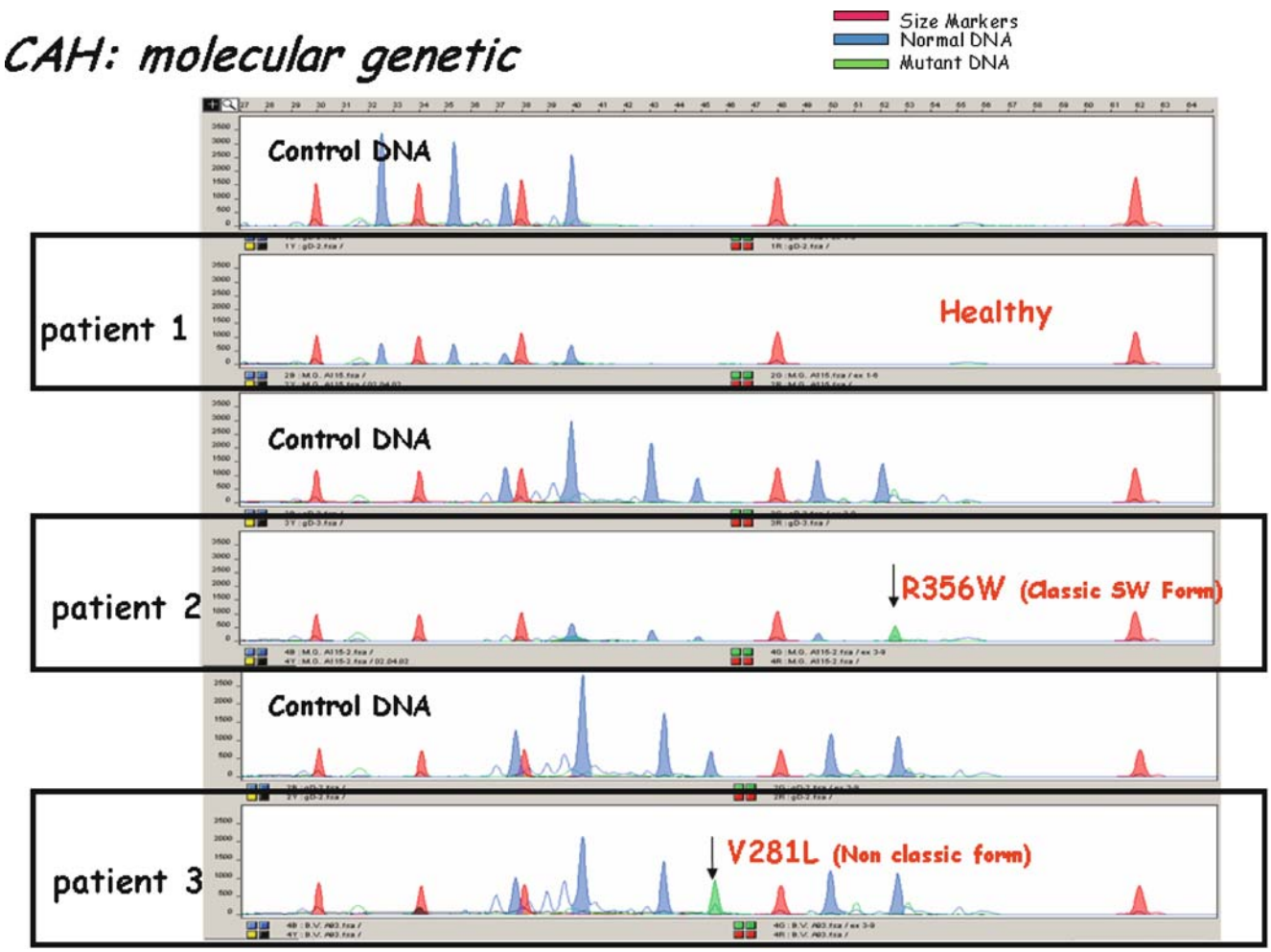


**Fig. 8** Output of LDR reaction in normal DNA (control) and three patients: patient 1 was a 13-year-old girl with signs of hyperandrogenism and a borderline 17-hydroxyprogesterone (20 nmol/L) after ACTH injection. Patient 2 was a newborn boy at term with 670 nmol/L 17-hydroxyprogesterone at screening. Patient 3 was a term-born girl who had basal mildly elevated 17-hydroxyprogesterone at screening (64 nmol/L) and after ACTH (88 nmol/L). The molecular genetic assay excluded a CAH in patient 1 and confirmed classic CAH in patients 2 and 3

*Additional methods*

*Minisequencing* (Krone et al 2002) is based on the same principle as LDR, with a second DNA-polymerase step instead of the ligase reaction.

The *amplicon melting curve* method is based on analysis of the melting curve of amplified DNA fragments (amplicons). DNA melts at characteristic melting temperatures ($T_m$) that are defined as the temperatures where half of the helical structure of the DNA is lost. These structural changes can be assayed by capillary electrophoresis of the target fragments at increasing temperatures (melting curve). The detection is based on fluorescent tags (e.g. SYBR Green). Since the differences in $T_m$ are directly dependent on the nucleotide composition of the DNA, melting curve analysis allows distinction between DNA fragments of different composition. (for more information http://www.roche-applied-science.com). As for LDR, this technique allows detection of known mutations only.

*Sequencing* for the detailed analysis of the whole sequence of the CYP21A1 gene is still the only genetic analysis with 100% detection rate, but its use is not yet broadly established.

## Genotype–phenotype correlation

*CYP21* mutations can be grouped into three categories according to the level of enzymatic activity predicted from *in vitro* mutagenesis and expression studies. The first group consists of so-called *null* mutations (deletions or nonsense mutations) that totally abolish enzyme activity; these are most often associated with salt-wasting disease (Speiser and White 2003). The second group of mutations, consisting mainly of the missense mutation Ile172Asn (I172N) yields enzymes

with reduced activity (1–2% of normal). These mutations permit adequate aldosterone synthesis and are associated with simple virilizing disease. The final group includes mutations such as Val281Leu (V281L) and Pro30Leu (P30L) that produce enzymes retaining 20–60% of normal activity; these mutations are associated with the nonclassic disorder.

When the 21-OHD phenotype is quantitated with the use of 17-OHP levels or scores for signs of androgen excess or salt wasting, a phenotype–genotype correlation of 80–90 % is found. Compound heterozygotes for two different *CYP21* mutations usually have a phenotype compatible with the presence of the milder of the gene defects (Speiser et al 1992).

## Treatment

CAH is a chronic disease that requires long-term therapy. In the classic form of the disease glucocorticoids are required not only to overcome the cortisol deficiency mostly in stress situations, but also to suppress the ACTH-driven stimulation of andrenal androgens. In 75% of the cases, mineralocorticoids substitution is required and salt supplementation is advisable in infancy. Surgical correction of the ambiguous genitalia in girls is also part of the management of these patients. It is not recommended to treat infants and children affected by the nonclassic form of the disease until symptoms and signs of androgen excess become evident. For more details on this topic, see Speiser and White (2003).

## Conclusions

Recent advances in the diagnostic procedure of CAH have dramatically improved the management of the disease, not only in the early phases of life thanks to the newborn screening but also in the prenatal period thanks to reliable genetic analysis.

By using gestational age-related cut-off values in newborn screening the number of unwanted recalls, particularly in premature infants, has been significantly reduced. The advent of tandem mass-spectrometry has opened further possibilities for more specific and targeted analysis for the screening and beyond.

Finally, a genetically well-characterized disease such as 21-hydroxylase deficiency might eventually be a target for gene therapy. Since this therapeutic approach is still costly in time and investment, the selection of the patients and implementation of such an approach for this as well as other metabolic diseases must be further improved.

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